PATENT
Attorney Docket No. UCSD-07017

AMENDMENTS TO THE SPECIFICATION PURSUANT TO REVISED 37 CFR § 1.21

Please insert the following paragraph between the "Title" and the "Background" sections on page one of the application as filed on February 15, 2001:

> This application claims priority to U.S. provisional application Serial No. 60/182,685, filed on February 15, 2000 that, while pending at the time the instant non-provisional was filed, is now abandoned.

Please replace paragraph [0037] with the following paragraph:

> [0037] FIG. 1. Induction of CTL against hTRT in peripheral blood leucocytes (PBMC) from normal blood donors. T cells from HLA-A2+~~BLA-A2+~~ individuals were stimulated by autologous PBMC pulsed with hTRT-derived synthetic peptides as detailed in the Material and Methods. (A). Results refer to effector cells from individual donors immunized in vitro against p540 (SEQ ID NO:01). Open circles define T2 cells and closed circles T2 cells pulsed with p540 (SEQ ID NO:01) as targets. (B). Results refer to effector cells from individual donors immunized in vitro against p865 (SEQ ID NO:02). Open diamonds define T2 cells and closed diamonds T2 cells pulsed with p865 (SEQ ID NO:02) as targets. Effector to target ratios are indicated on an individual basis. Percent cytotoxicity was calculated as specified in The Materials and Methods.

Please replace paragraph [0043] with the following paragraph:

> [0043] As used herein, the terms "telomerase" and "telomerase complex" refer to functional ~~functional~~ telomerase enzymes. It is intended that the terms encompass the complex of proteins found in telomerases. For example, the terms encompass the 123 kDa and 43 kDa telomerase protein subunits.

Please replace paragraph [0044] with the following paragraph:

[0044] Telomerase is a ribonucleoprotein enzyme which has been linked to malignant transformation in human cells. Telomerase activity is increased in the vast majority of human tumors making its gene product the first molecule common to all human tumors. The generation of endogenously-processed telomerase peptides bound to Class I major histocompatibility complex (MHC) molecules could therefore target cytotoxic T lymphocytes (CTL) to tumors of different origins. This could advance vaccine therapy against cancer provided that precursor CTL recognizing telomerase peptides in normal adults and cancer patients can be expanded through immunization. Applicant demonstrates here that the majority of normal individuals and patients with prostate cancer immunized in vitro against two HLA-A2.1~~HLA-A2.1~~ restricted peptides (p540 set forth as SEQ ID NO:1; and p865 set forth as SEQ ID NO:2) from telomerase reverse transcriptase (hTRT), develop hTRT specific CTL. This suggests the existence of precursor CTL for hTRT in the repertoire of normal individuals and in cancer patients. Most importantly, cancer patients' CTL specifically lysed a variety of HLA-A2+ cancer cell lines, demonstrating immunological recognition of endogenously-processed hTRT peptides. Moreover, in vivo immunization of HLA-A2.1 transgenic mice generated a specific CTL response against both hTRT peptides. Based on the induction of CTL responses in vitro and in vivo, and the susceptibility to lysis of tumor cells of various origins by hTRT CTL, Applicant suggests that hTRT could serve as a universal cancer vaccine for humans.

Please replace paragraph [0055] with the following paragraph:

[0055] PBMC were separated by centrifugation on Ficoll-Hypaque gradients and plated in 24-well plates at 5.times.105 cells/ml/well in RPML-1640 supplemented with 10% human AB+ serum, L-glutamine and antibiotics (CM). Autologous PBMC (stimulators) were pulsed with hTRT synthetic peptides p540 or p865 (10 .mu.g/ml) for 3 hours at 37 .degree. C. Cells were then irradiated at 5000 rads, washed once, and added to the responder cells at a responder:stimulator ratio ranging between 1:1 and 1:4. The next day, 12 IU/ml ~~IL-2~~ IL-2 (Chiron Co., Emeryville, Calif.) and 30 IU/ml IL-7 (R&D Systems, Minneapolis, Minn.) were added to the cultures. Lymphocytes were re-stimulated weekly with peptide-pulsed autologous adherent cells as follows. First, autologous PBMC were

incubated with hTRT peptide (10 .mu.g/ml) for 3 hours at 37 .degree. C. Non-adherent cells were then removed by a gentle wash and the adherent cells were incubated with fresh medium containing the hTRT peptide (10 .mu.g/m) for an additional 3 hours at 37.degree. C. Second, responder cells from a previous stimulation cycle were harvested, washed and added to the peptide-pulsed adherent cells at a concentration of 5.times.10^5 cells/ml (2 ml/well) in medium without peptide. Recombinant IL-2 and IL-7 were added to the cultures on the next day.

Please replace paragraph [0063] with the following paragraph:

[0063] The amino acid sequence of hTRT (locus AF015950) (19) was analyzed for 9mer peptide sequences containing known binding motifs for the HLA-A2.1 molecule [52; 35; 60], a subtype encompassing 95% of HLA-A2 allele which is expressed in about 50% of the Caucasian population (26-28). Peptides were identified by reverse genetics based on canonical anchor residues for HLA-A2.1 (29), and by using the software of the Bioinformatics & Molecular Analysis Section (NIH) available at [http://bimas.dcrt.nih.gov/molbio/hla_bind/index.html] the corresponding NIH web site which ranks 9mer peptides on a predicted half-time dissociation coefficient from HLA Class I molecules (30). From an initial panel of ~30 candidate peptides Applicant retained two sequences, 5401LAKFLHWL548 (SEQ ID NO:1) and 865RLVDDFLLV873 (SEQ ID NO:2), denoted hereunder as p540 and p865.

Please replace paragraph [0069] with the following paragraph:

[0069] Whether or not CTL against hTRT could also be induced in cancer patients was studied in four HLA-A2.1+~~LA-A2.1+~~ individuals with clinical and histological diagnosis of prostate cancer. All four patients were refractory to hormonal therapy, three had metastases and none had prostatectomy. In prostate cancer, the most common cause of cancer in men, high hTRT expression has been documented in 84% of cases (34). Marked lysis of peptide-pulsed T2 cells was observed in 3 out of 4 individuals after three rounds of in vitro stimulation (FIGS. 2,A and B). Both peptides yielded comparable CTL responses in all three individuals with maximal lysis ranging between 27-49% and 48-52%, respectively. CTL against both peptides lysed LnCap, a HLA-A2.1+ prostate cancer cell line, with maximal lysis ranging between 24-36% for p540 and 12-40% for p865.

Prostate cancer cell line PC-3, which is HLA-A2.1-, was used as control and was not lysed (FIG. 2,C). Both prostate cancer cell lines tested positive for hTRT by the TRAPeze (telomerase detection assay; INTERGEN)(not shown), suggesting that the CTL generated against the synthetic peptides might lyse cancer cells by recognizing hTRT peptide/MHC Class I complex at the surface of cancer cells.

Please replace paragraph [0082] with the following paragraph:

[0082] The peptide selection was confirmed using the application available online at the web site of the Bioinformatics & Molecular Analysis Section of the NIH [[(http://bimas.dcrt.nih.gov/molbio/hla_bind/index.html)]] that ranks potential 9 mer peptides based on a predicted half-time dissociation from HLA class I molecules deduced from (58). In our pilot studies one of the peptides identified using the "manual"[[--]]approach--P865--(SEQ ID NO:02) ranked among the top 5 HLA-A2-binding peptides identified through the software-guided analysis. Another peptide--P540--(SEQ ID NO:01) ranked at the top in the software-guided analysis.

Please replace paragraph [0086] with the following paragraph:

[0086] Unlike p540, which was characterized as having a high affinity binding (slow half time dissociation) to HLA-A2 (Table VII), these peptides have an estimated half time dissociation score faster than prototype p540. Calculations were made using the program [[([http:]//bimas.dcrt.nih.gov/molbio/hla_bind/index.html)]] on the "bimas" section of the NIH web site.

Please replace paragraph [0095] with the following paragraph:

[0095] In conclusion, based on the demonstration that precursor CTL specific for two hTRT peptides can be expanded in patients with cancer, their CTL recognize the same hTRT peptides on tumor cells of various origins and histological types, and a strong *in vivo* CTL response against both hTRT peptides was induced in HLA-A2.1+~~BLA-A2.1+~~ monochain transgenic mice, Applicant suggests that hTRT can be regarded as a universal cancer antigen and its peptides as the substrate for a possible universal cancer vaccine for humans.

Please insert the following paragraph on a separate page after the "Claims" section of the application as filed on February 15, 2001:

ABSTRACT

The present invention concerns vaccines effective for treating cancer. This invention particularly concerns a universal cancer vaccine involving telomerase reverse transcriptase as a specific tumor antigen, a method for its use for targeting cytotoxic T lymphocytes to tumor cells, and a method for induction and/or augmentation of a cancer patient's immune response against his tumor.